

HIV-1VAU and HIV-1LAI gp120 sequences (SEQ ID NO:46 and SEQ ID NO:48) and HIV-1VAU and HIV-1LAI gp41 sequences (SEQ ID NO:47 and SEQ ID NO:49).

Figure 4 is a multiple alignment of the immunodominant peptides in the extracellular segment of the transmembrane envelope glycoprotein of various HIV-1 isolates (SEQ ID NOs:50-62).

Figures 5A-B contain branches of a phylogenetic tree.

Figure 6 is the DNA sequence of HIV-1VAU env gene (SEQ ID NO:63).

Figure 7 is the DNA sequence of HIV-1VAU virus integrase gene (SEQ ID NO:64).

Figures 8A-C contain a comparison of the GAG amino acid sequence of various HIV-1 strains (SEQ ID NOs:65-70).

Figure 9A is a multiple alignment of the V3 loop of gp120 (SEQ ID NOs:71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, and 93).

Figure 9B is a multiple alignment of the immunodominant region of gp41 (SEQ ID NOs:72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, and 94).

Figure 10A is a 513 base pair DNA sequence of the GAG region of HIV-1DUR (SEQ ID NO:95).

Figure 10B is the corresponding 171 amino acid sequence of Figure 10A (SEQ ID NO:96).

Figure 11A is a 525 base pair DNA sequence of the V3 loop region of gp120 of HIV-1DUR (SEQ ID NO:97).

Figure 11B is the corresponding 175 amino acid sequence of Figure 11A (SEQ ID NO:98).

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Figure 12A is a 312 base pair DNA sequence of the immunodominant region of gp41 of HIV-1DUR (SEQ ID NO:99).

Figure 12B is the corresponding 104 amino acid sequence of Figure 12A (SEQ ID NO:100).

Figure 13A shows specific primer sequences for HIV-1 type O (SEQ ID NOs:42-45).

Figure 13B shows the corresponding positions of the primers of Figure 13A in different HIV type O strains.

Figures 14A-B are serological correlation results.

Figures 15A-C are nucleotide comparisons of HIV sequences.

Figures 16A-C are protein comparisons of HIV sequences.

Figure 17 is a phylogenetic tree.

Figure 18A-B show an HPLC chromatogram of a VAU peptide.

Figure 19A-B show a mass spectroscopy analysis of a VAU peptide (SEQ ID NO:101).

Figures 20A-C contain immunoreactivity data.

Figures 21A-C contain immunoreactivity data --

Page 8, beginning at line 26 through page 9, line 2, replace this paragraph with the following text:

-- Two weeks after coculturing the patient's CD8-depleted, PHA-stimulated PBMCs with similar cells from a healthy donor, the production of virus was detected in the form of an RT activity peak in the culture supernatant. This virus could then be subjected to serial passages on CD8-depleted, PHA-stimulated normal PBMCs. Figure 1A represents the production of HIV-1_(VAU) in infected PBMC culture supernatants, checked by RT assay (filled circles) and HIV-1 p24 antigen capture ELISA (empty circles). The concentration of HIV-1 p24 is expressed in ng/ml and the RT activity in

cpm/ μ l. In Figure 1B, the same experiment was carried out with a standard primary HIV-1 isolate from an AIDS patient. --

Page 11, beginning at line 14 through line 20, replace this paragraph with the following text:

-- The invention relates to any variant of the nucleic acid sequences of the HIV-1_(VAU) virus or of any group O equivalent virus, containing structural proteins which have the same immunological properties as the structural proteins coded for by the env gene comprising the sequence described in Figure 6 and called "vau", also designated by SEQ ID NO:63. --

Page 11, beginning at line 33 through page 12, line 11, replace this paragraph with the following text:

-- The invention relates to the DNAs or DNA fragments, more particularly cloned DNAs and DNA fragments, obtained from RNA, cDNA or primers which can be used in PCR, or other gene amplification methods, derived from the HIV-1_(VAU) retrovirus RNA or DNA. The invention relates more particularly to all the equivalent DNAs, especially to any DNA having sequence homologies with the HIV-1_(VAU) DNA, in particular with the sequence coding for the env region of the HIV_(VAU) strain comprising the sequence corresponding to SEQ ID NO:63 represented in Figure 6 and called "vau". The homology with HIV-1 group M is at least equal to 50%, preferably to 70% and still more advantageously to about 90%. Generally, the invention relates to any equivalent DNA (or RNA) capable of hybridizing with the DNA or RNA of a group O HIV-1 retrovirus. --

Page 12, beginning at line 14 through line 18, replace this paragraph with the following text:

--The invention also relates to the HIV-1_(VAU) integrase gene comprising the sequence identified by the same SEQ ID NO:64 or hybridizing with SEQ ID NO:64. The invention also relates to the RNAs corresponding to the DNA described above. --

Page 13, beginning at line 19 through page 14, line 11, replace this paragraph with the following text:

-- The resulting amplification product was cloned into a pBluescript vector, generating the clone ph4, deposited at the Collection Nationale des Cultures de Micro-organismes (CNCM), Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15 France, on 20 October 1994 under No. I-1486, which was subsequently used as a probe to screen a lambda library of low molecular weight DNA, which was digested with EcoRI and was obtained from cells infected with HIV-1_(VAU). Briefly, the PBMCs infected

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with HIV-1_(VAU) were cocultured for 24 hours with new PBMCs stimulated with PHA and depleted of CD8⁺ cells, after which a high cytopathic effect (CPE) was visible. The low molecular weight DNA was then extracted according to the Hirt method (Hirt 1967), and digested with the enzyme EcoRI. A previous Southern-blot analysis of this DNA had indeed shown that the HIV-1_(VAU) genome contained only one EcoRI site, permitting the cloning of nonintegrated circular DNA species representing the entire viral genome. The resulting digestion product was subjected to agarose gel electrophoresis, and the population of DNA fragments of approximately 8-12 kb in size was purified and ligated to EcoRI-digested lambda Zap DNA (Stratagene). After encapsidation, plating and screening by hybridization with ³²P-labeled ph4 DNA, a clone, λH34, was identified as being positive, and amplified. The EcoRI insert was purified, sonicated, and cloned by the "shotgun" technique into the phosphatase-treated vector M13mp18 digested with the enzyme SmaI. One hundred and fifty of the clones obtained were sequenced in a 373A DNA sequencer (Applied Biosystems), and the resulting sequences were assembled into a single sequence using the Wisconsin GCG DNA analysis package. --

Page 14, beginning at line 24 through line 36, replace this paragraph with the following text:

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-- The PCR amplification was carried out in 35 thermal cycles at 92°C for 15 seconds, 52°C for 1 minute, 60°C for 2 minutes and 72°C for 2 minutes. The resulting amplification product, of 3.5 kb in size, was cloned into the M13mp18 vector and sequenced by successive reactions, first using the M13 universal sequencing primer, and then the primers deduced from the upstream sequences. Analysis of the nucleotide and peptide sequences was carried out using the Wisconsin GCG DNA analysis package. The HIV-1_(VAU) *env* gene codes for 877 amino acids in total, including the signal peptide. The nucleotide sequence of the HIV-1_(VAU) *env* gene corresponds to SEQ ID NO:63 (see Figure 3). --

Page 15, beginning at line 29 through line 37, replace this paragraph with the following text:

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-- Particularly advantageous are the probes which, when hybridized with HIV-1, give a strong reaction with HIVs belonging to group O and weak reaction with HIVs belonging to group M. By way of nonlimiting example, a probe constructed from the HIV-1_(VAU) virus integrase gene sequence SEQ ID NO:64 gives, when it is hybridized with HIV-1 under hybridization conditions such as those described in Patent EP 178 978, a strong reaction with group O HIVs and a weak reaction with group M HIVs. --

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Page 17, beginning at line 13 through line 23, replace this paragraph with the following text:

-- The invention relates to an external envelope protein of the HIV-1_(VAU) retrovirus encoded by the gene comprising the sequence corresponding to SEQ ID NO:63. According to a preferred embodiment of the invention, this protein is in addition characterized in that it comprises the amino acid sequence corresponding to SEQ ID NO:46 represented in Figure 3 and comprising amino acid residues 1 to 526. The subject of the invention is also any polypeptide or variant which is derived from said sequence having an epitope which may be recognized by the antibodies induced by the HIV-1_(VAU) virus. --

Page 17, beginning at line 26 through line 31, replace this paragraph with the following text:

-- The subject of the invention is also an envelope transmembrane protein comprising the amino acid sequence SEQ ID NO:47 represented in Figure 3 between amino acid residues 527 and 877. This transmembrane protein is, within the scope of the invention, in glycosylated or nonglycosylated form. --

Page 19, beginning at line 7 through line 17, replace these paragraphs with the following text:

-- Preferred polypeptides of this region are, for example, those which contain the sequence CKNRLIC (SEQ ID NO:5) or correspond to this sequence. They may also be peptides or polypeptides corresponding to the sequence RLLALETFIQNWWLLNLWGCKNRLIC (SEQ ID NO:6) or comprising this sequence.

Another preferred peptide, identified below by the name "VAU peptide", corresponds to the following sequence or comprises this sequence or any part of this sequence capable of being recognized by antibodies directed against the HIV-1_(VAU) retrovirus RARLLALETFIQNQQLNLWGCKNRLICYTSVKWNKT (SEQ ID NO:7). --

Page 19, beginning at line 24 through line 31, replace this paragraph with the following text:

-- The present invention relates to a peptide obtained from the HIV-1-O DUR virus deposited on 23 February 1995 at the Collection Nationale des Cultures de Micro-organismes (CNCM), Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15 France, under the reference I-1542, or a peptide whose sequence is distinguished from that of the above by substitution, deletion or addition of amino acids, this separate peptide nevertheless retaining the antigenic characteristics of the above one. --

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Page 19, beginning at line 34 through page 21, line 9, replace these paragraphs with the following text:

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-- Thus, a preferred peptide of the invention is a peptide containing at least 4 consecutive amino acids contained in the GAG sequence represented in Figure 8A-C or in an immunologically similar GAG sequence obtained from a variant of the HIV-1-O DUR virus, said immunologically similar sequence being recognized by antibodies which also specifically recognize at least one of the sequences AHPQQA (SEQ ID NO:8), LWTTTRAGNP (SEQ ID NO:9) contained in the GAG sequence of Figure 8.

Preferably, this peptide consists of a peptide whose amino acid sequence is contained either in one of the following sequences:

SPRTLNAWVKAVEEKAFNPEIIPMFMALEGA (1) (SEQ ID NO:10)

MLNAIGGHQGALQVLKEVIN (2) (SEQ ID NO:11)

GPLPPGQIREPTGSDIAGTTSTQQEQI (3) (SEQ ID NO:12)

IPVGDIYRKWIVLGLNKMVKMYSPPVSLDI (4) (SEQ ID NO:13)

QGPKEPFRDYVDRFYKTKLAE (5) (SEQ ID NO:14)

AHPQQA (5a) (SEQ ID NO:8)

LWTTTRAGNP (5b) (SEQ ID NO:9)

or in a corresponding immunologically similar sequence, this peptide containing at least four consecutive amino acids of one of said sequences.

Preferably also, this peptide consists of a peptide whose amino acid sequence is contained either in one of the following sequences:

SPRTLNAWVK (6) (SEQ ID NO:15)

GSDIAGTTST (7) (SEQ ID NO: 16)

QGPKEPFRDYVDRF (8) (SEQ ID NO: 17)

or in a corresponding immunologically similar sequence, this peptide containing at least four consecutive amino acids of one of said sequences.

Peptides which are particularly preferred in the present invention are the peptides containing:

-the amino acid sequence NPEI (9) (SEQ ID NO:18)

or

- the amino acid sequence AVEEKAFNPEIIPMFMA (10) (SEQ ID NO:19), and more particularly peptides whose amino acid sequence is contained, either in one of the following sequences:

IGGHQGALQ (23) (SEQ ID NO:20)

REPTGSDI (24) (SEQ ID NO:21)

or in a corresponding immunologically similar sequence, this peptide containing at least 4 consecutive amino acids of one of said sequences, as well as the peptide whose amino acid sequence is contained, in the following amino acid sequence:

IDEAADWD (25) (SEQ ID NO:22)

or in a corresponding immunologically similar sequence, this peptide containing at least four consecutive amino acids of said sequence. --

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Page 21, beginning at line 20 through line 33, replace this paragraph with the following text:

-- A peptide derived from the HIV-1-O DUR virus defined above also falls within the scope of the present invention, said peptide containing at least 4 consecutive amino acids of the V3 loop of gp120 represented in Figure 9A or of the corresponding immunologically similar sequence, obtained from a variant of the HIV-1-O DUR virus, said immunologically similar sequence being recognized by antibodies which also specifically recognize at least one of the sequences:

KEIKI (12) (SEQ ID NO:23)
 EREGKGAN (13) (SEQ ID NO: 24)
 CVRPGNNSVKEIKI (14) (SEQ ID NO:25)
 QIEREGKGANSR (15) (SEQ ID NO:26). --

Page 21, beginning at line 35 through page 22, line 14, replace this paragraph with the following text:

-- This peptide preferably contains:

- a) either the sequence CVRPGNNSVKEIKIGPMAWYSMQIEREGKGANSRTAFC (11) (SEQ ID NO: 27) or a part of this sequence which contains at least 4 amino acids
- b) or an amino acid sequence which is separate from the sequence of a) in which one or more amino acids are replaced with one or more amino acids, with the proviso that the peptide retains its reactivity with an antiserum against the abovementioned peptide,
- c) or an amino acid sequence which is separate from a) or b), in which one or more amino acids have been deleted or added, with the proviso that the peptide retains its reactivity with an antiserum against the peptide of a),
- d) or a corresponding immunologically similar sequence or part of a sequence. --

Page 22, beginning at line 16 through page 23, line 5, replace these paragraphs with the following text:

-- Preferably also, this peptide contains either the sequence KEIKI (12) (SEQ ID NO:23), or the sequence EREGKGAN (13) (SEQ ID NO:24), or the sequence GPMWYSM (16) (SEQ ID NO:28).

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In a particularly preferred manner, a peptide as defined above contains the amino acid sequence CVRPGNNSVKEIKI (14) (SEQ ID NO:25) or the sequence QIEREGKGANSR (15) (SEQ ID NO:26).

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A peptide derived from the HIV-1-O DUR virus as defined above also falls within the scope of the invention, said peptide containing at least 4 consecutive amino acids, whose entire sequence is contained in the sequence of the immunodominant region of gp41 represented in Figure 9B or in a corresponding immunologically similar sequence, obtained from a variant of the HIV-1-O DUR virus, said immunologically similar sequence being recognized by antibodies which also specifically recognize at least one of the following sequences:

RLLALETLMQNQQL (17) (SEQ ID NO:29),
LNLWGCRGKAICYTSVQWNETWG (18) (SEQ ID NO:30),
CRGKAI (19) (SEQ ID NO:31),
SVQWN (20) (SEQ ID NO:32),
RLLALETLMQNQQLLNLWGCRGKAICYTS (21) (SEQ ID NO:33),
QNQQLLNLWGCRGKAICYTSVQWN (22) (SEQ ID NO:34).

✓ Page 23, beginning at line 7 though line 27, replace this paragraph with the following text:

-- This peptide is preferably a peptide containing the sequence RLLALETLMQNQQL (17) (SEQ ID NO:29) or LNLWGCRGKAICYTSVQWNETWG (18) (SEQ ID NO:30) or part of this peptide (18) (SEQ ID NO:30) containing:

- B 17
- a) either the sequence CRGKAI (19) (SEQ ID NO:31) or the sequence SVQWN (20) (SEQ ID NO:32) in which Q is, where appropriate, replaced by a different amino acid, which is nevertheless also different from K, or the two sequences at the same time,
 - b) or an amino acid sequence which is separate from the sequence of a) in which one or more amino acids are replaced with two amino acids, with the proviso that the peptide retains its reactivity with an antiserum against the peptide of a),
 - c) or an amino acid sequence which is separate from a) or b), in which one or more amino acids have been deleted or added, with the proviso that the peptide retains its reactivity with an antiserum against the peptide of a),
 - d) or in a corresponding immunologically similar sequence or part of a sequence. --

Page 23, beginning at line 29 through page 24, line 4, replace this paragraph with the following text:

-- Preferably also, this peptide possesses one or the other of the following characteristics:

- its N-terminal sequence which contains at least 8 amino acids is not immunologically recognized by antibodies formed against the sequence RILAVERY

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(SEQ ID NO:35) contained in the immunodominant region of gp41 of the HIV-1-LAI strain.

- it is not recognized by antibodies formed against the peptide SGKLIC (SEQ ID NO:36) of the HIV-1-LAI strain.

- it contains either of the following two sequences:

RLLALETLMQNQQLLNWGCGRGKAICYTS (21) (SEQ ID NO:33)

QNOQLLNWGCGRGKAICYTSVQWN (22) (SEQ ID NO: 34). --

Page 25, beginning at line 9 through line 25, replace this paragraph with the

following text:

-- The peptide is checked by HPLC and by mass spectrometry according to the electrospray technique (Figures 18A-B and Figures 19A-B) (FISON VG Trio 2000 spectrophotometer).

Fmoc: 9-Fluorenylmethyloxycarbonyl

Pmc: 8-Methylpentane-6-sulfonylchroman

Trt: Trityl

Boc: Tertbutyloxycarbonyl

tBU: tert butyl

DMF: Dimethylformamide

DIPCDI: Diisopropylcarbodiimide

HOBT: 1-Hydroxybenzotriazole

TF: Trifluoroacetic acid

Reagent K: Phenol/water/thioanisole/ethanedithiol/TFA: 2.5 ml/2.5 ml/2.5 ml/1.5 ml/41 ml

Comparison of the amino acid sequence of the HIV-1_(VAU) envelope with the corresponding sequence of other HIV viruses. --

Page 26, beginning at line 31 through page 27, line 9, replace this paragraph

with the following text:

-- The alignment of Figure 3 shows numerous regions of high divergence, with a few domains retained here and there. These retained regions correspond roughly to the domains also retained in the conventional HIV-1 isolates (Alizon et al. 1986, Benn et al. 1985). Among the divergent domains, the V3 loop, also called principal determinant of neutralization (Javaherian et al. 1990, Javaherian et al. 1989, Matsushita et al. 1988) is clearly one of the most divergent, although the two cysteines defining the loop are retained. The sequence of the cap of the loop, GPGRAF (SEQ ID NO:37) for HIV-1-LAI is GPMAYWY (SEQ ID NO:38) in HIV-1_(VAU). This unit of the cap is identical to that of the Cameroonian group O isolate HIV_(ANT70) (Van den Heasevelde et al. 1994), but is different from that of the other group O isolate, HIV_(MVP5180) (Gürtler et al. 1994), for which the motif is GPMRWR (SEQ ID NO:39). --

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Page 30, beginning at line 30 through page 31, line 16, replace this paragraph with the following text:

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-- Generally, the invention relates to any composition which can be used for the in vitro detection of the presence, in a biological fluid, especially from individuals who have been brought into contact with HIV-1_(VAU), or with antibodies against at least one of the HIV-1_(VAU) antigens. This composition can be applied to the selective diagnosis of infection by an HIV-1 group O by using diagnostic techniques such as those described in Patent Applications EP 84401834 and EP 87400,151,4. Within the context of the present invention, any constituent comprising antigenic determinants capable of being recognized by antibodies produced against HIV-1_(VAU) is used, for example recombinant antigens or peptides or chemically synthesized peptides defined from the sequence of the HIV-1_(VAU) envelope. In this regard, the invention relates more particularly to compositions containing at least one of the HIV-1_(VAU) virus envelope proteins. There may be mentioned, by way of examples of compositions, those which contain proteins, glycoproteins or peptides from the envelope protein corresponding to the entire 590-620 region of the HIV-1_(VAU) gp41 protein or to the parts of this region which are specific for HIV-1_(VAU) such as the peptides -TFIQN- (SEQ ID NO:40) or -WGCKNR- (SEQ ID NO:41). --

Page 37, beginning at line 21, through page 38, line 13, replace this paragraph with the following text:

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-- The experimental data collated in the two tables of Figures 20A-C and 21A-C show that:

- a) the four sera taken from patients contaminated with the HIV-1 group (or subgroup) O virus are very reactive with the vau peptide;
- b) the ten sera supposedly taken from patients contaminated with the HIV-1 group (or subgroup) O virus, among the 19 sera sent out by the Pasteur Institute of Yacoundé, are also highly reactive with this same peptide;
- c) the sera (4 samples) taken from individuals contaminated with the HIV-1 subtype B virus (in the acute phase) are not reactive with the vau peptide;
- d) the sera taken from asymptomatic blood donors (48 samples tested) are not reactive with the vau peptide; These experimental data, although limited (in view of the paucity of HIV-1 group (or subgroup O) antibody-positive samples), bear witness to the sensitivity and specificity of the peptide selected. --

Page 40, beginning at line 10 through line 16, replace this paragraph with the following text:

-- According to the present invention, the process of detection and discrimination between infection by an HIV-1 group (or subgroup) O retrovirus and an HIV-1 subgroup

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M retrovirus is characterized by placing serum, obtained from individuals subjected to an AIDS diagnostic test, in contact, in particular, with the peptide RILAVERY (SEQ ID NO:35). --

Page 45, beginning at line 19 through line 26, replace this paragraph with the following text:

-- Oligonucleotide primers also according to the invention have a sequence consisting of at least eight consecutive nucleotides of the following nucleotide sequences:

ATT CCA ATA CAC TAT TGT GCT CCA-3' (SEQ ID NO:42)

AAA GAA TTC TCC ATG ACT GTT AAA-3' (SEQ ID NO:43)

GGT ATA GTG CAA CAG CAG GAC AAC-3' (SEQ ID NO:44)

AGA GGC CCA TTC ATC TAA CTC-3' (SEQ ID NO:45).

Page 47, beginning at line 13 through Page 48, line 3, replace these paragraphs with the following text:

-- Using the VAU sequence and its correlation with the MVP5180 and ANT70 sequences, oligonucleotide primers were defined which endeavor to be specific for the subgroup O in its entirety for the V3 region and the gp41 region. These primers made it possible to amplify the DUR strain and consequently constituted one solution to the amplification problem encountered. The position and the sequence of these HIV subgroup O primers are represented in Figure 13B and A respectively. These primers make it possible to obtain an amplification band which is visible on staining with ethidium bromide, with a single step of 30 cycles of PCR. Partial sequences were obtained:

- GAG: 513 base pairs (171 amino acids) = SEQ ID NO:95 and SEQ ID NO:96, Figure 10A and B
- gp120 V3 loop: 525 base pairs (75 amino acids) = SEQ ID NO:97 and SEQ ID NO:98, Figure 11A and B
- gp41 immunodominant region: 312 base pairs (104 amino acids) = SEQ ID N:99 and SEQ ID NO:100, Figure 12A and B.

Nucleotide (Figure 15A-C) and protein (Figure 16A-C) comparisons of the DUR sequences with the MVP5180, ANT and VAU sequences for the O subgroup, LAI for the HIV-1 consensus sequence, representative African HIV-1 MAL sequence, and CPZ for the CIV of the Gabonese chimpanzee, show that DUR is as remote from the other published HIV-1 group (or subgroup) O strains as the latter are from each other. --

Page 48, beginning at line 35 through page 49, line 2, replace this paragraph with the following text:

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